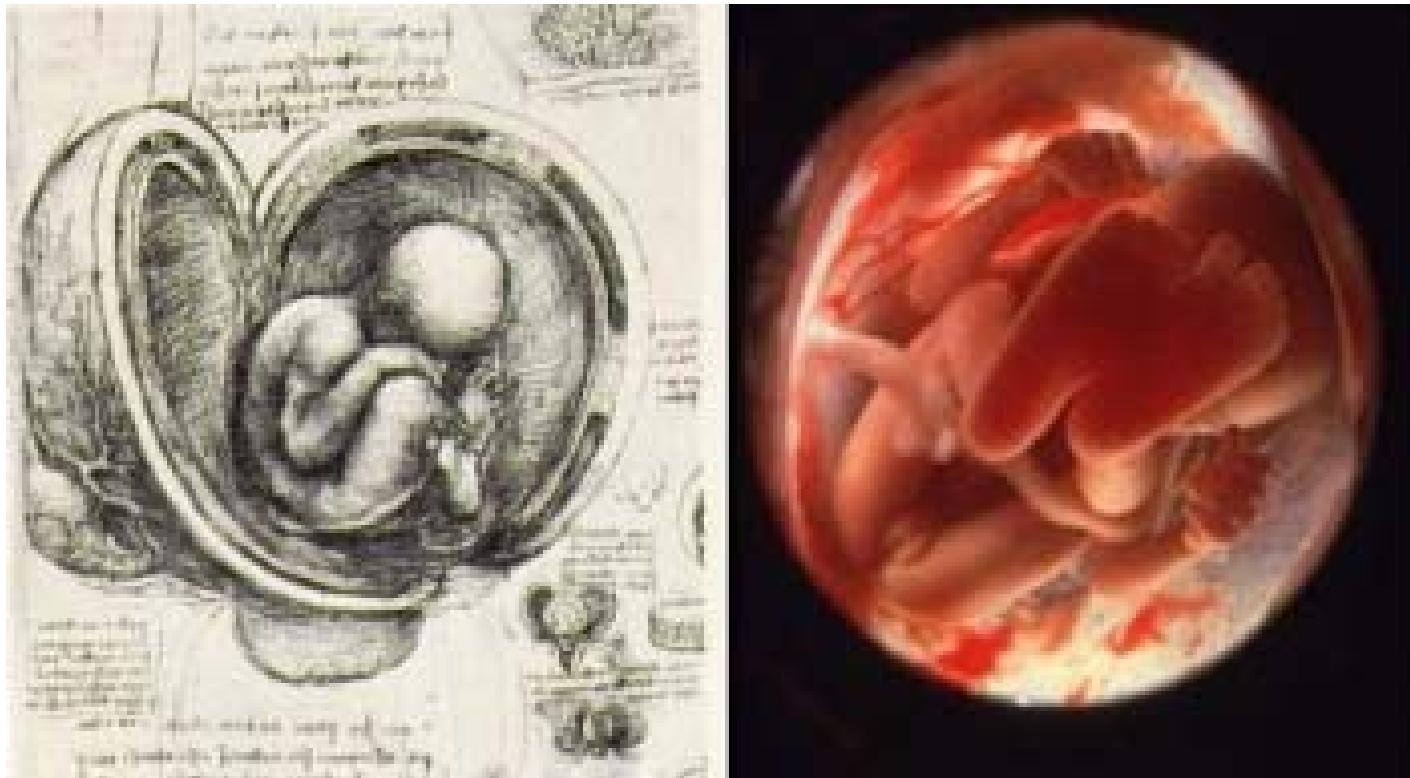


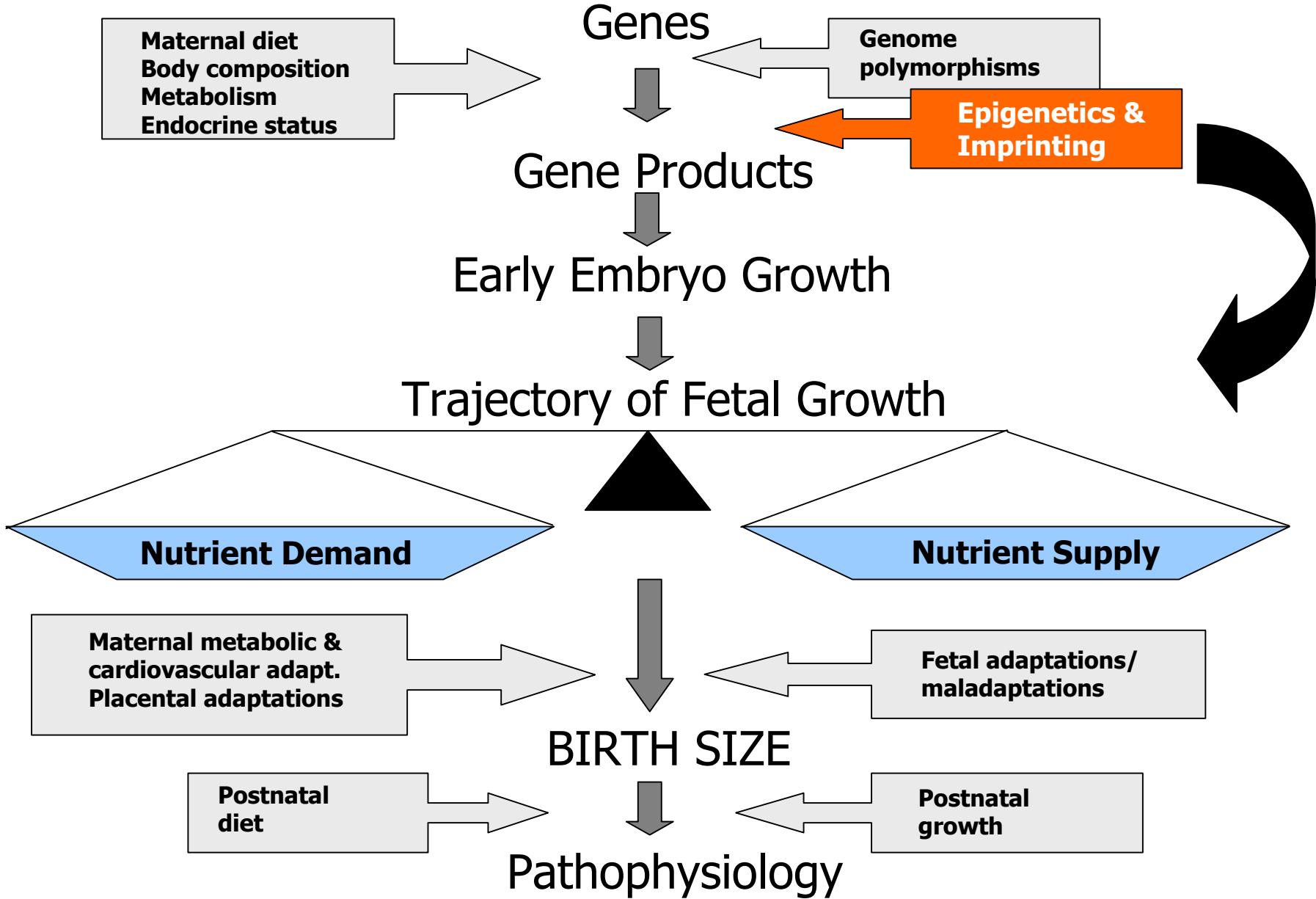
# Dr. Miguel Constancia

Hosted By:  
U.S. Department of Health and Human Services  
National Institutes of Health

# Function of imprinting at the fetal-maternal interface



# Growth Programming in mammals



Adapted from Bertram & Hanson (2001) BMB

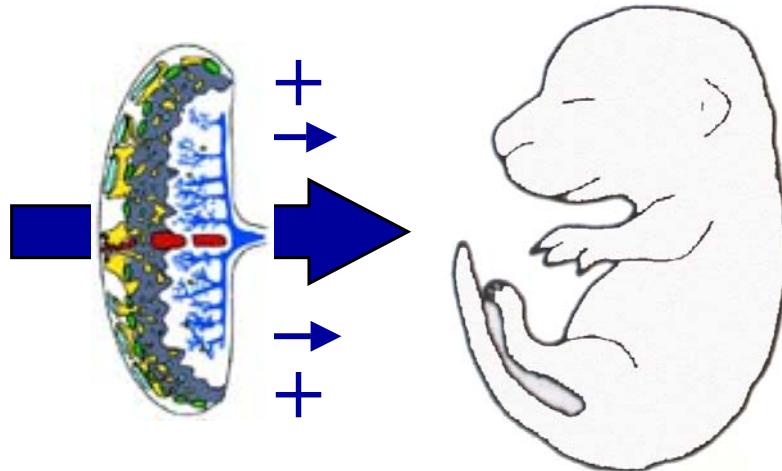
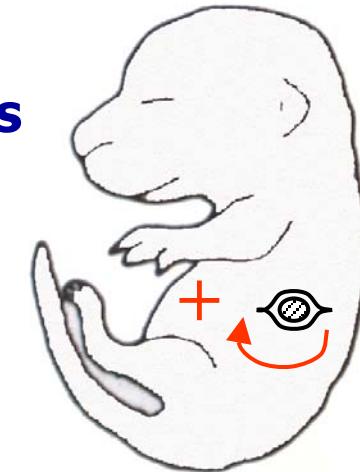
# Genetic control of supply and demand for nutrients

## Demand for nutrients

Expression of fetal growth factor



Cell growth and proliferation creates demand for nutrients

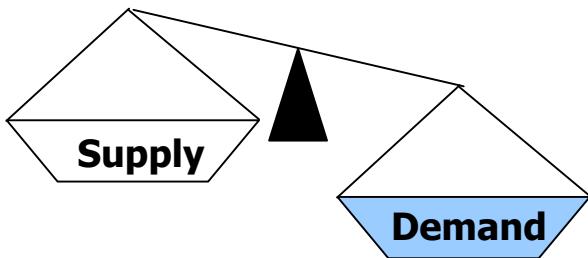
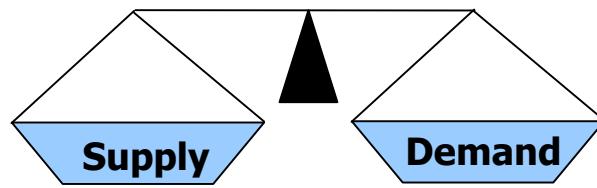
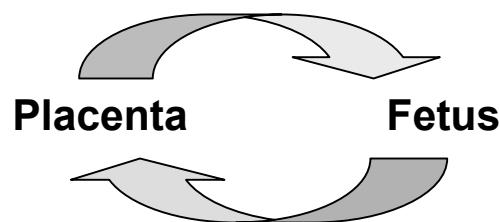
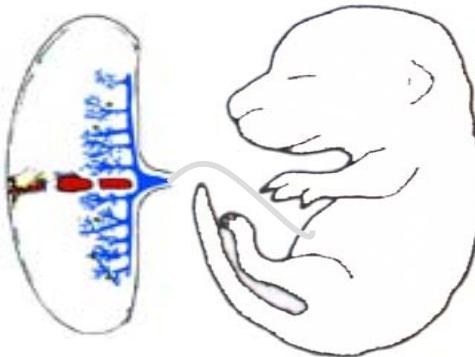


## Supply of nutrients

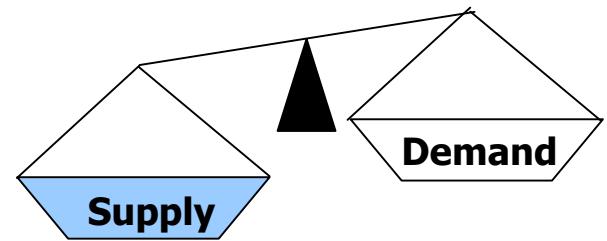
Expression of placental growth factor

Size and efficiency of placenta for nutrient transfer

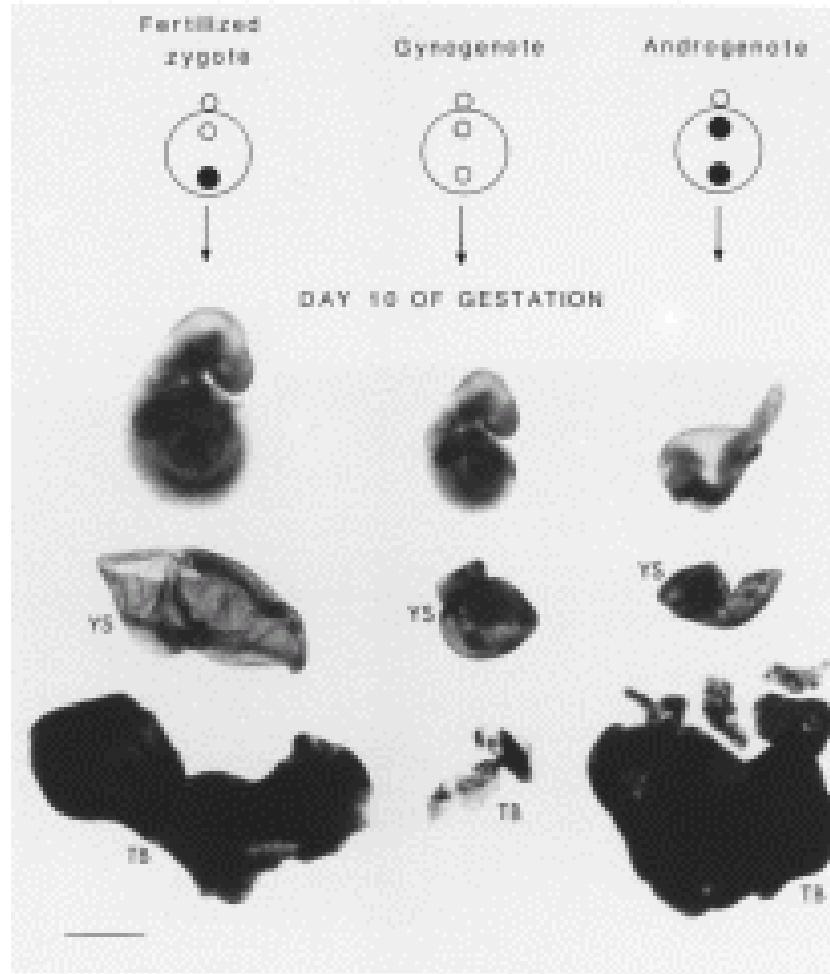
# Supply and demand signals



**Placental & Fetal  
adaptations**



# Genome-wide imprinting defects

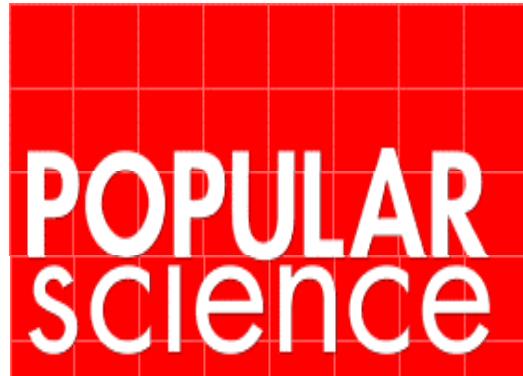


A. Surani & S. Barton

# Imprinted genes that affect growth

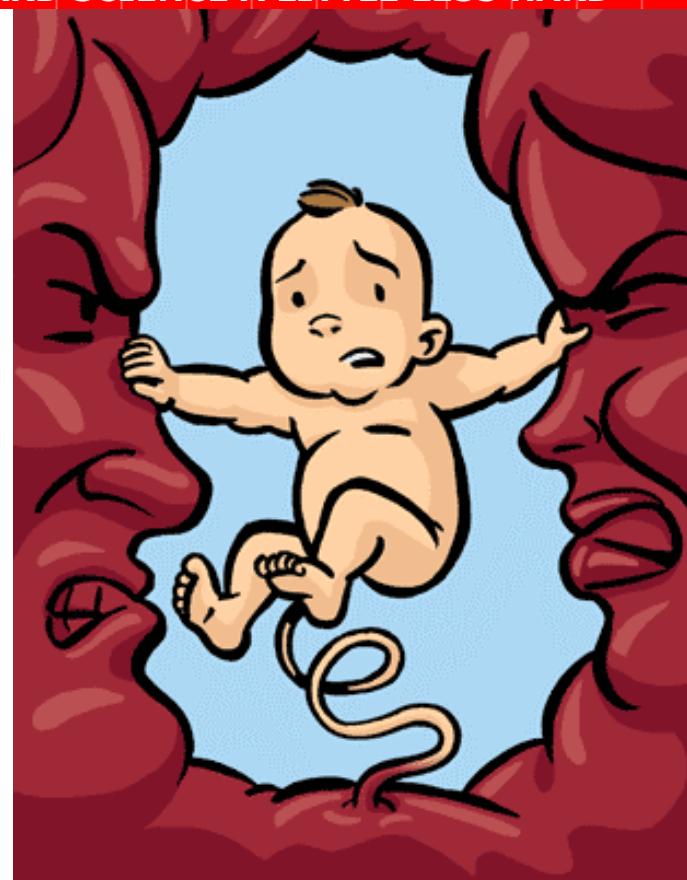
Gene	Loss of function in mice
<i>Ipl</i>	↑ placental growth
<i>Mash2</i>	placental differentiation - lethal
<i>Igf2r</i>	↑ fetal & placental growth - lethal
<i>Grb10</i>	↑ fetal growth
<i>Gnas/GnasXI</i>	↑ ↓ growth & post-natal behaviour; energy metabolism
<i>Cdkn1c</i>	↑ placental growth; proliferation defects - lethal
<i>Igf2</i>	↓ fetal & placental growth
<i>Peg1</i>	↓ fetal growth; nurturing
<i>Peg3</i>	↓ fetal growth; nurturing
<i>Rasgrf1</i>	↓ postnatal growth; long term memory

# Imprinting may exist because of 'genetic conflict'



THE POPSCI UNSCRAMBLER: MAKING  
HARD SCIENCE A LITTLE LESS HARD

Mom's and  
Pop's  
DNA Punch-up

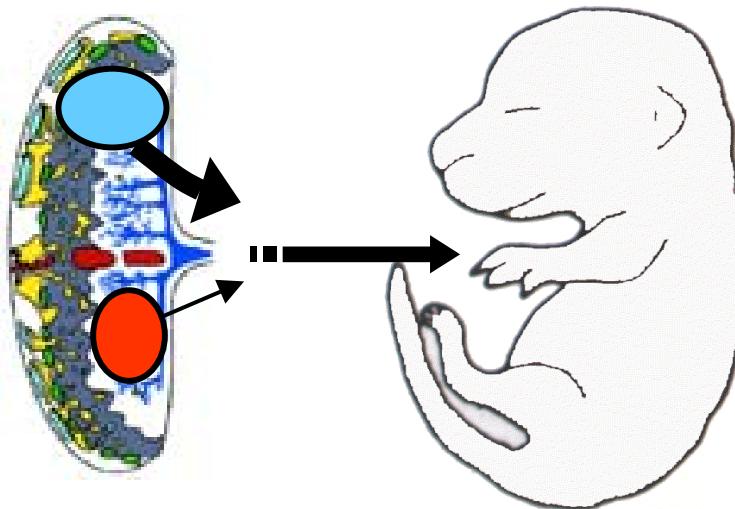


# **Genetic conflict hypothesis**

- Paternally expressed genes enhance fetal growth
- Maternally expressed genes suppress fetal growth

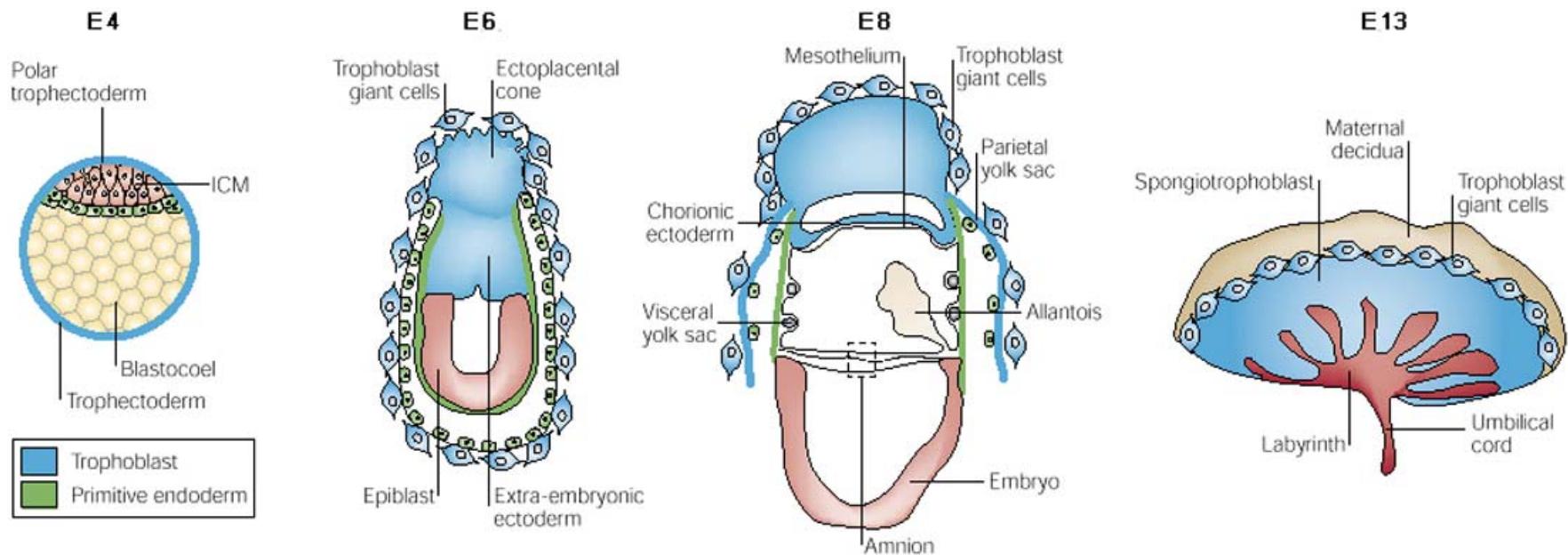
Paternal genes are selected to extract resources from the mother to give to the fetus, maternal genes are selected to inhibit this transfer of resources

# Function of imprinted genes in placenta and effects on fetal growth



- Paternal expression – enhanced nutrient transfer?
- Maternal expression – decreased nutrient transfer?
  - Knock-out mouse models
  - Placental transport assays

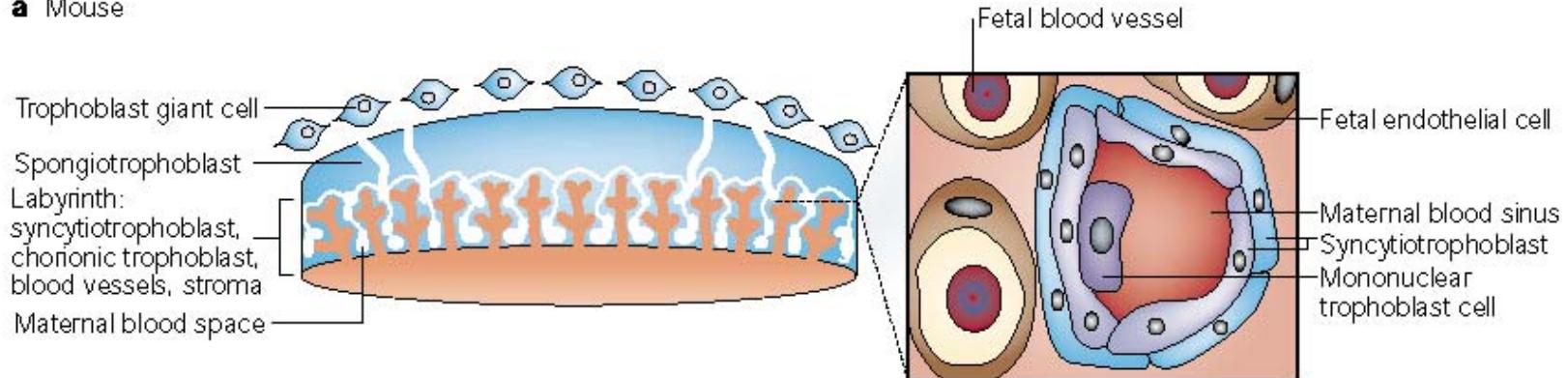
# Placental development in the mouse



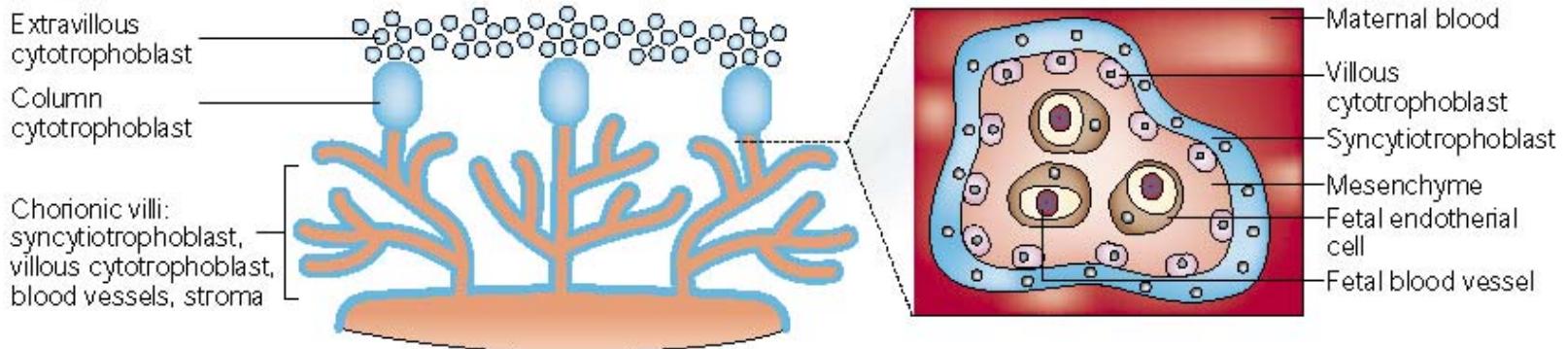
**J. Rossant & J. Cross**

# Comparative anatomy of the mouse and human placenta

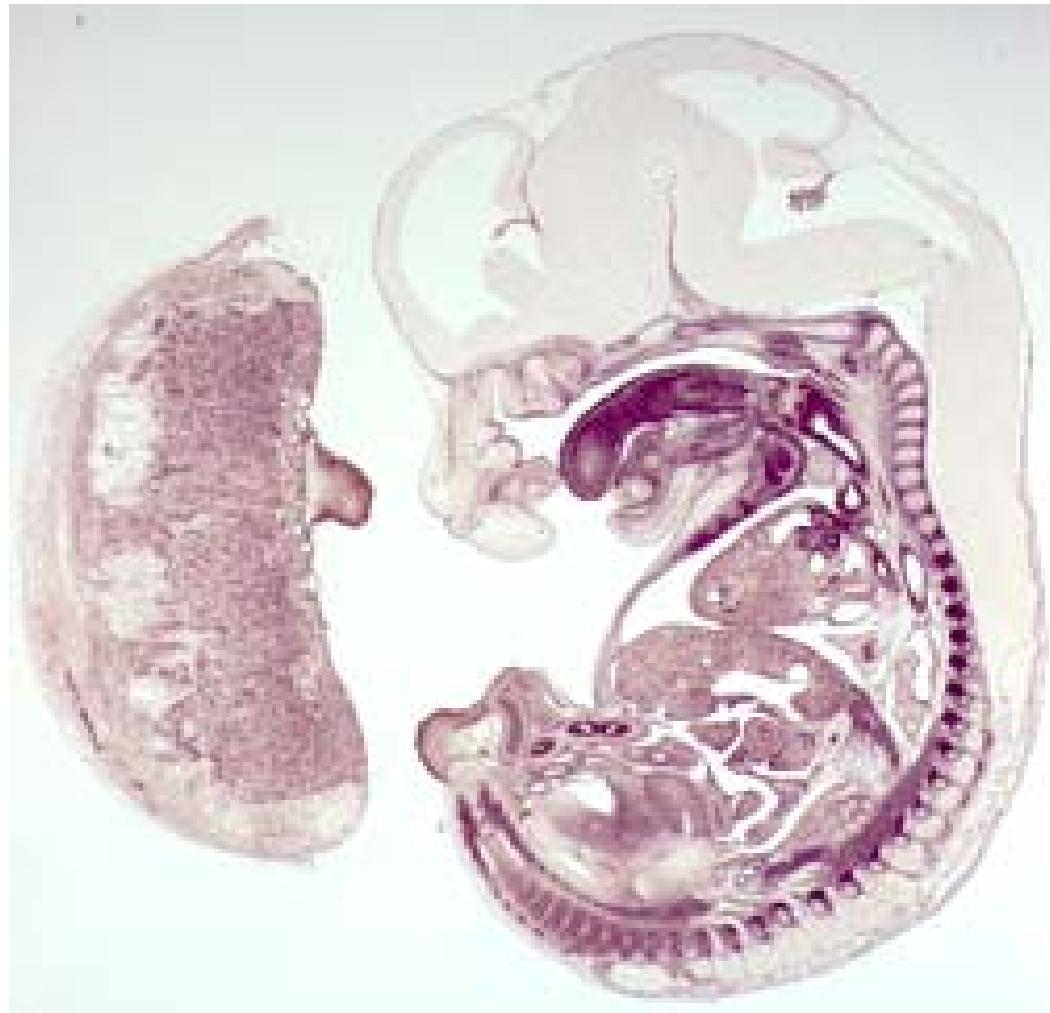
a Mouse



b Human

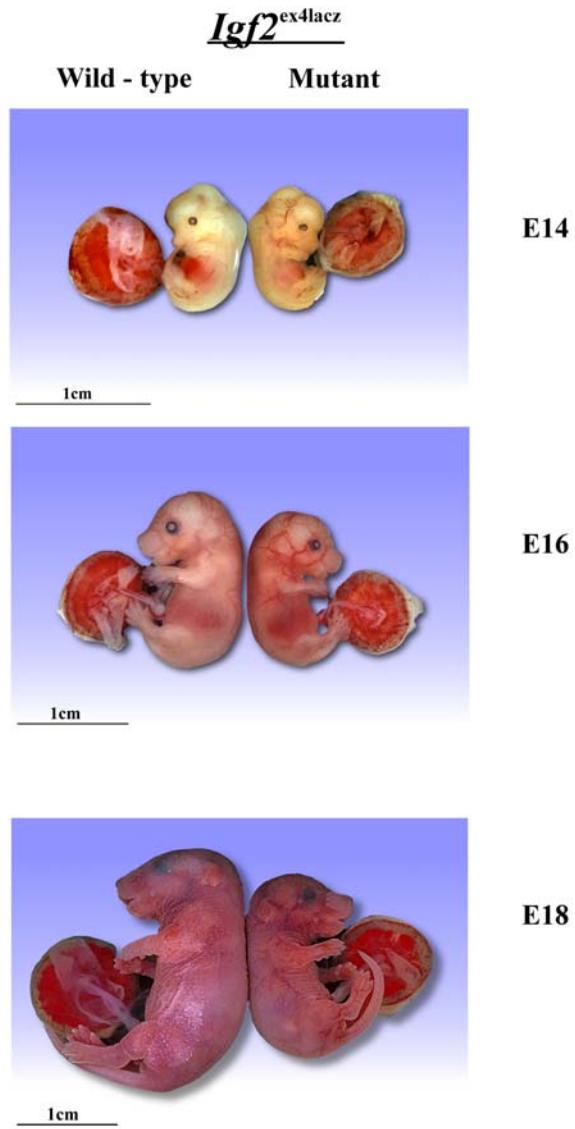


# *Igf2* gene as a model system to study supply and demand for maternal nutrients

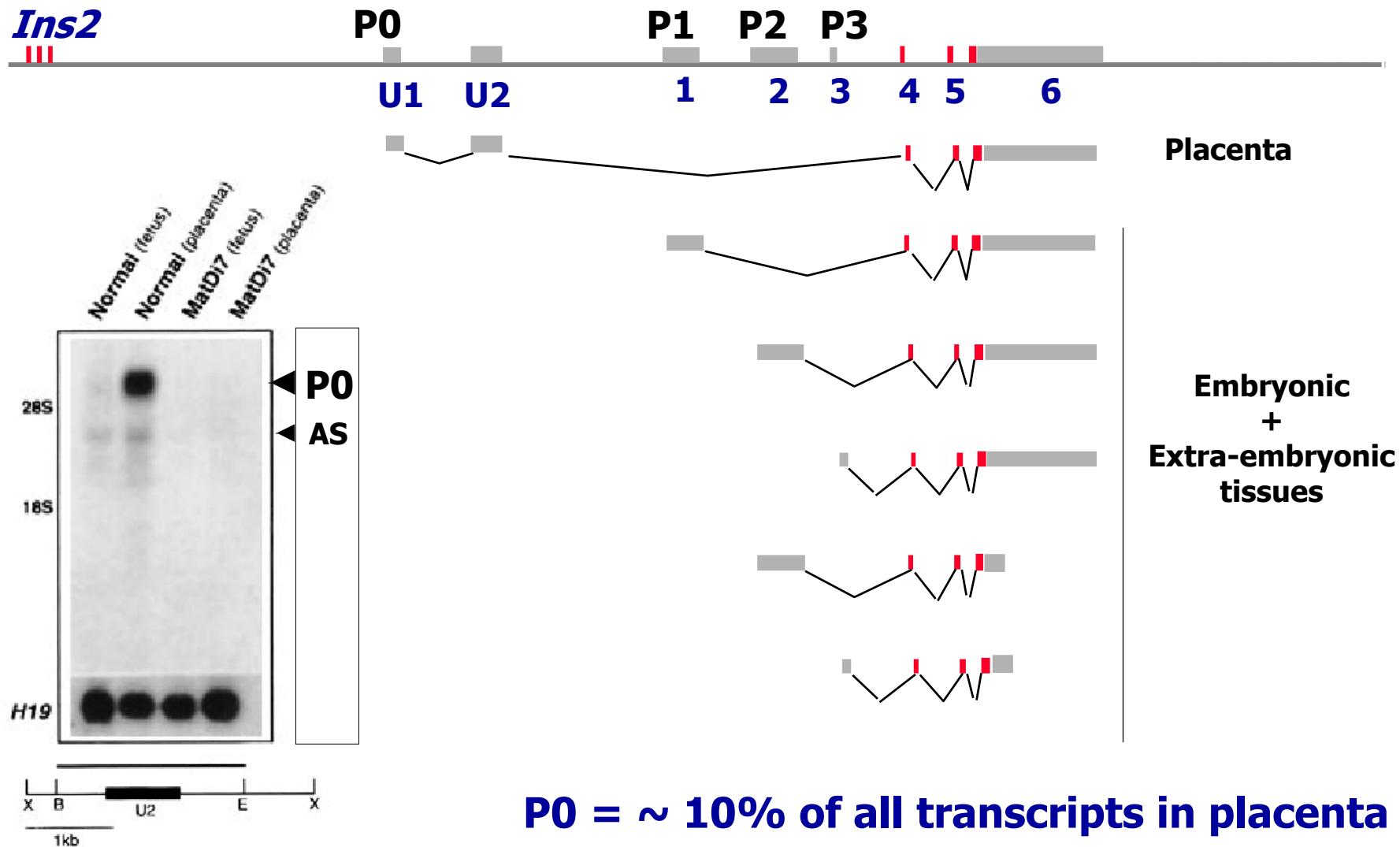


P. Smith & K. Davies

# *Igf2* null allele causes fetal and placental growth restriction



# Mouse *Igf2* gene



# *Igf2* P0 transcript is labyrinthine-trophoblast specific

E12



All *Igf2* transcripts

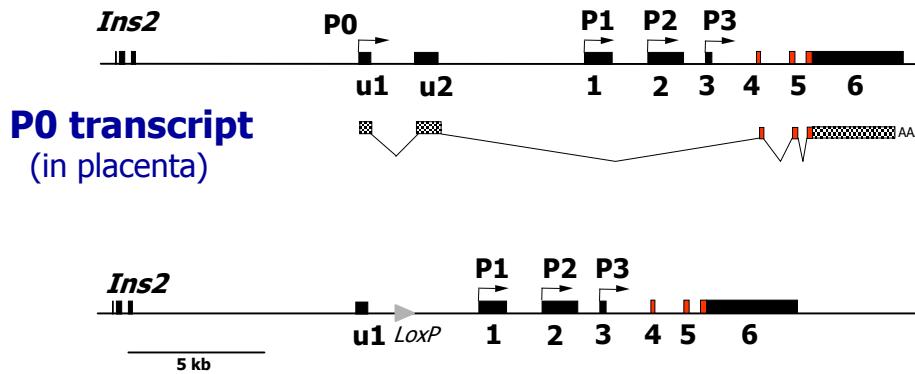


*Igf2* P0 transcript

Myriam Hemberger & Reinald Fundele

# The *Igf2*<sup>Δu2</sup> deletion

a



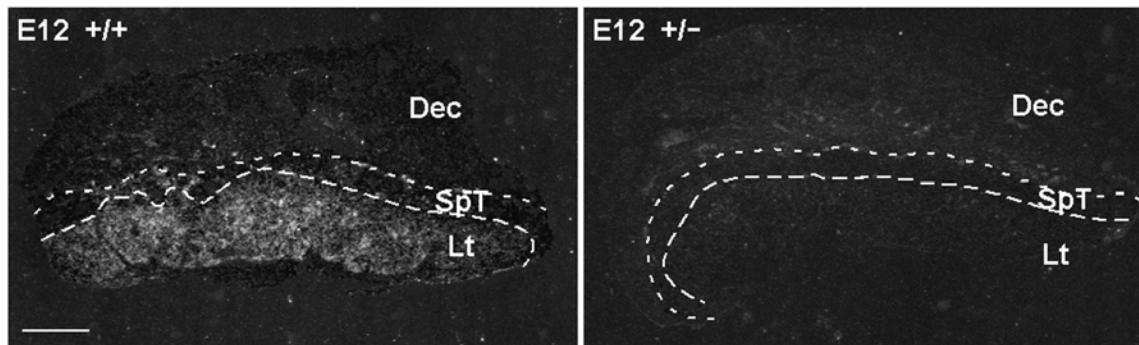
Wild-type *Igf2* allele

U2 knock-out allele

*Nat. Genet.* (2000) **94**:203-6

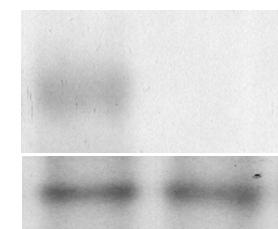
b

U2 probe



U1 probe

+/- +/–

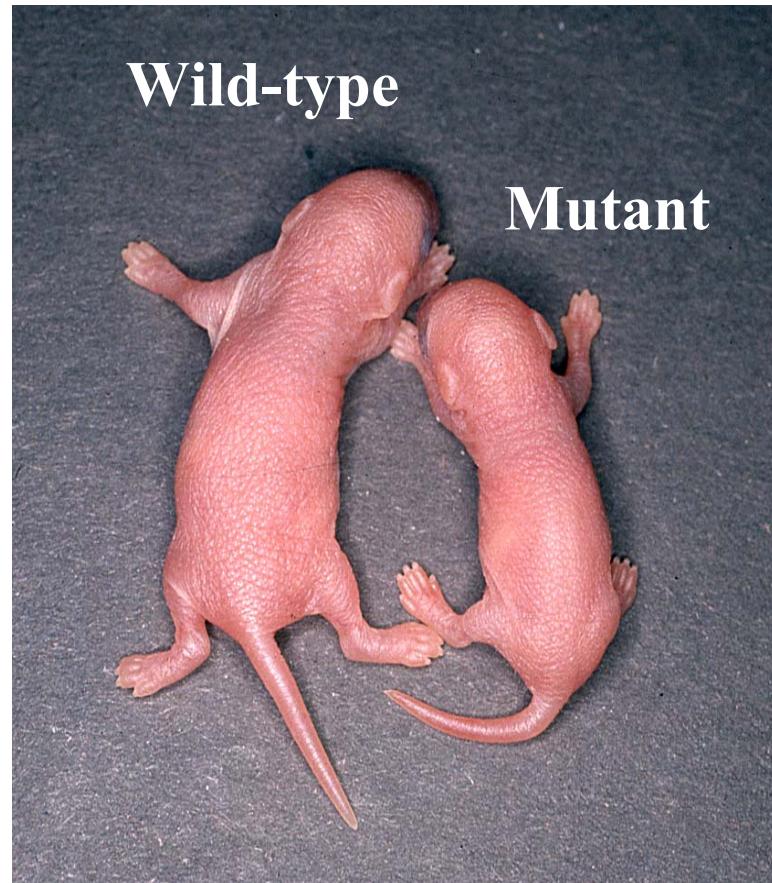


*Igf2* P0

control

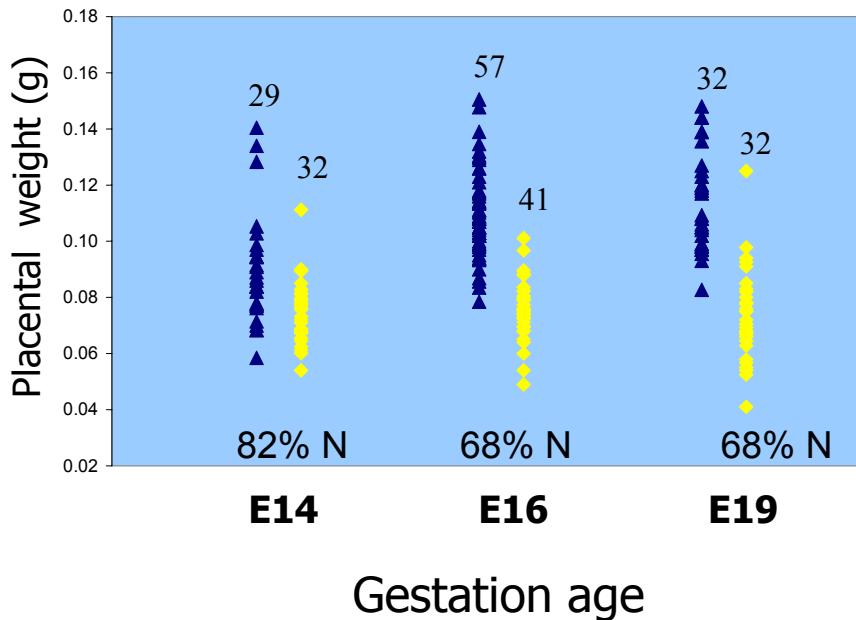
- U2 deletion is equivalent to a P0 promoter deletion

# *Igf2*<sup>ΔU2</sup> +/− mutants show a growth deficiency phenotype

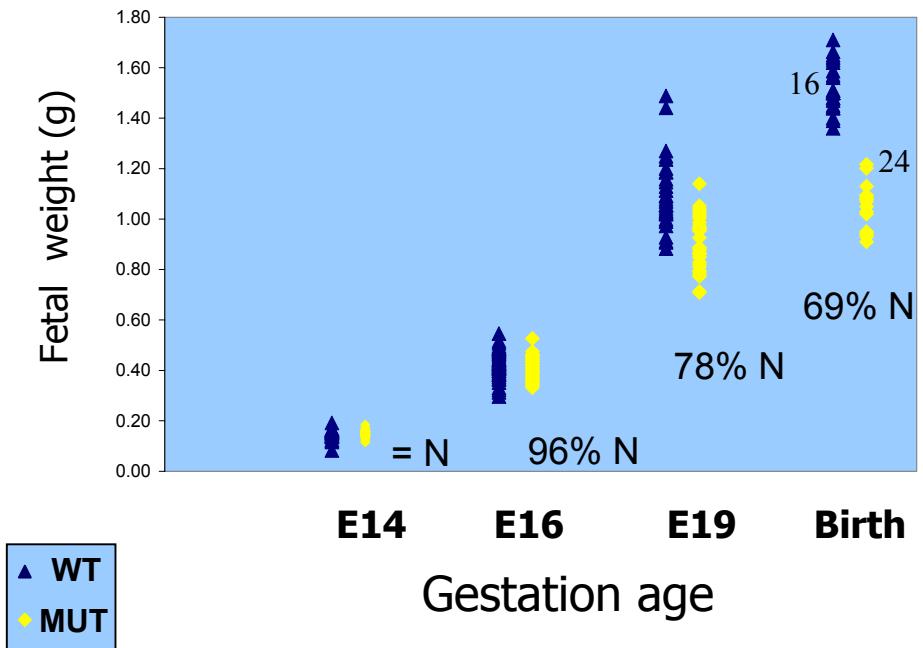


# Early placental and late fetal growth restriction in *Igf2<sup>ΔU2</sup>* +/- mutant mice

**A Placenta growth kinetics**



**B Fetal growth kinetics**



- Early placental growth effect

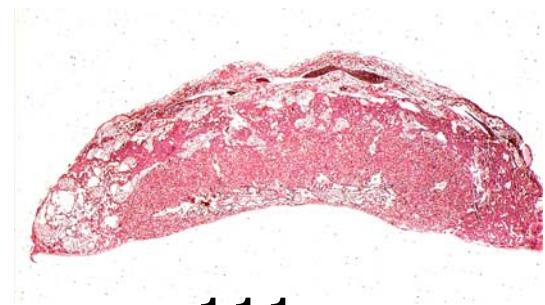
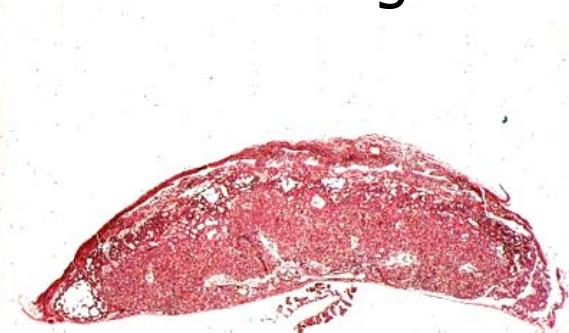
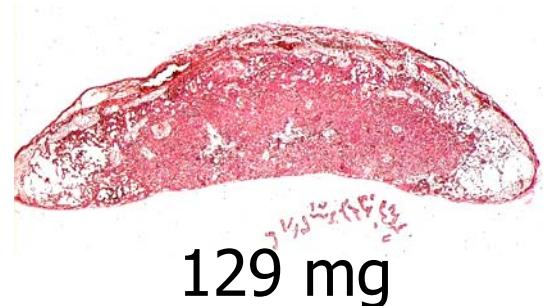
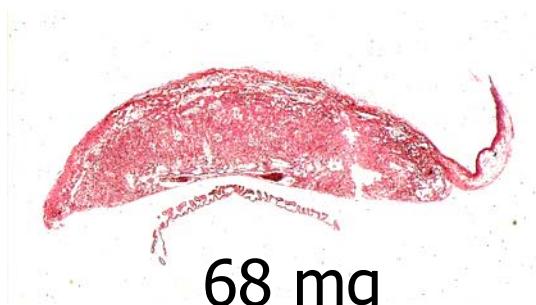
- Late fetal growth effect

# The placental growth deficiency in *Igf2<sup>ΔU2</sup>* mice is identical to that in *Igf2* null

*Igf2<sup>ΔU2</sup>* +/-

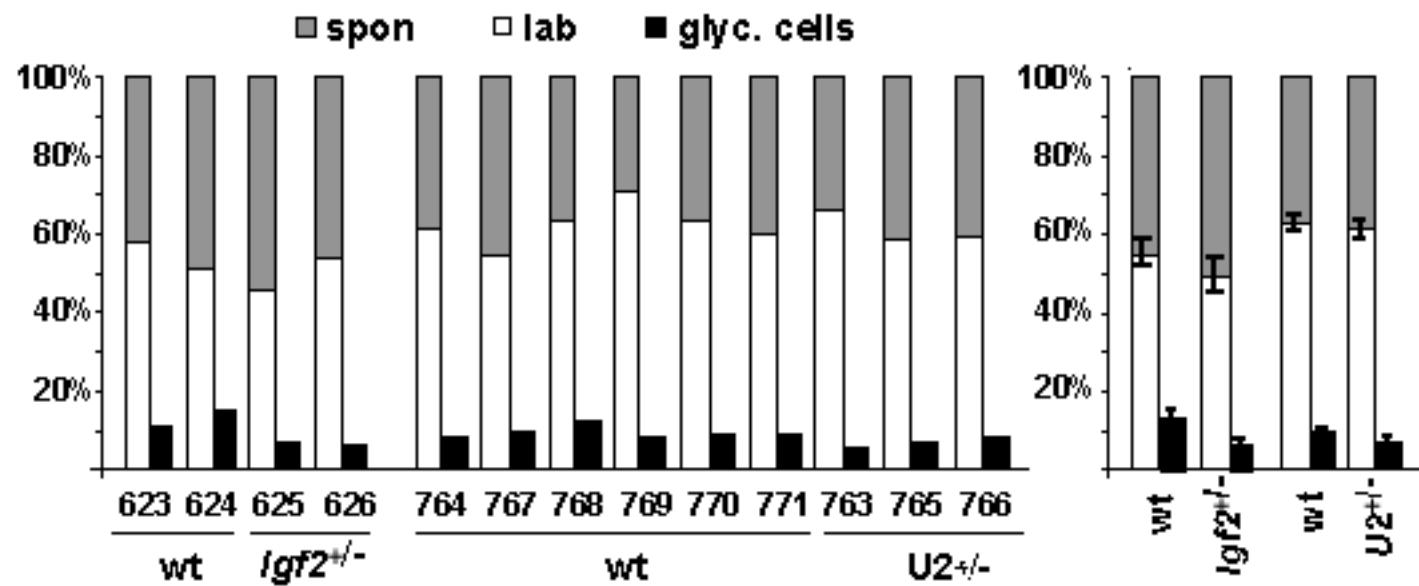
*Igf2* +/-

+/ +



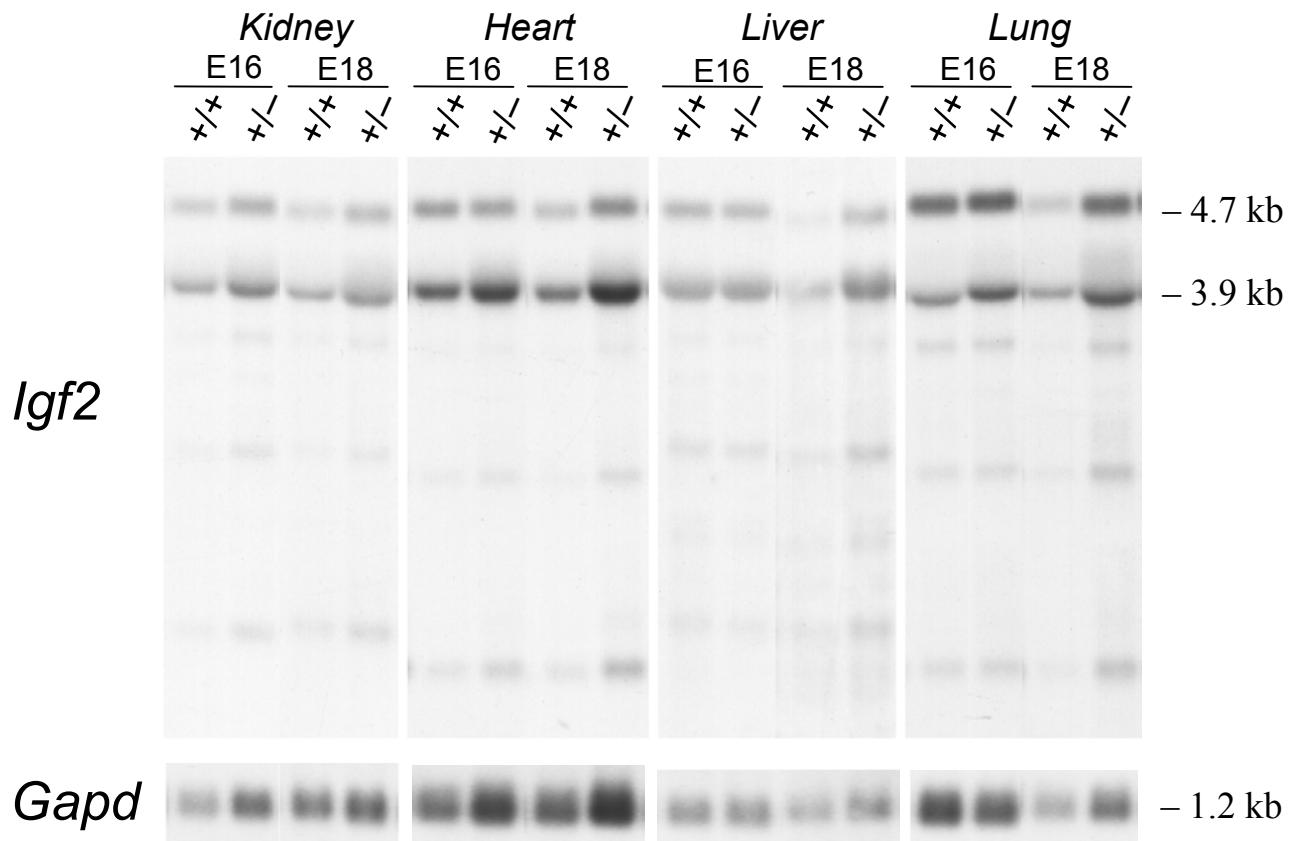
E16

# *Igf2<sup>ΔU2</sup>* and *Igf2* null placentas are proportionately smaller



# The *Igf2*<sup>Δu2</sup> deletion does not alter fetal *Igf2* levels

## Fetal tissues



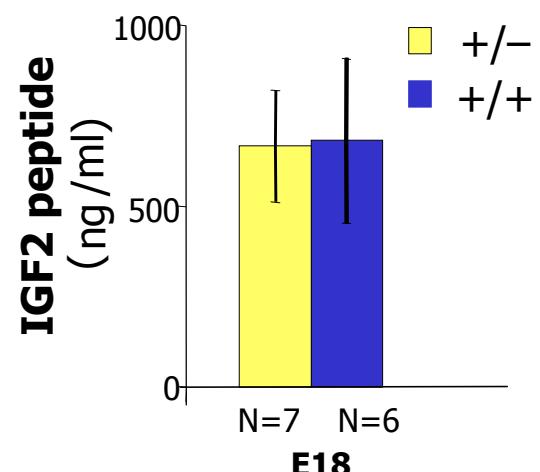
# The *Igf2*<sup>Δu2</sup> deletion eliminates specifically the P0 transcript

*Igf2* transcript levels

Organ	Gestational age	n	RNA ratio mut/wt
Placenta	E16	7mut; 5wt	1.10
	E18	6mut; 5wt	1.10
Liver	E16	6mut; 5wt	0.95
	E18	6mut; 6wt	1.30
Heart	E18	4mut; 6wt	1.00
Kidney	E18	6mut; 6wt	1.06

P>0.05

IGF2 serum levels



P>0.05

(David Hill, Canada)

# **Placental-specific IGF2 is a major modulator of placental and fetal growth**

Knockout of placental specific P0 transcript of *Igf2* results in placental and subsequent fetal growth restriction

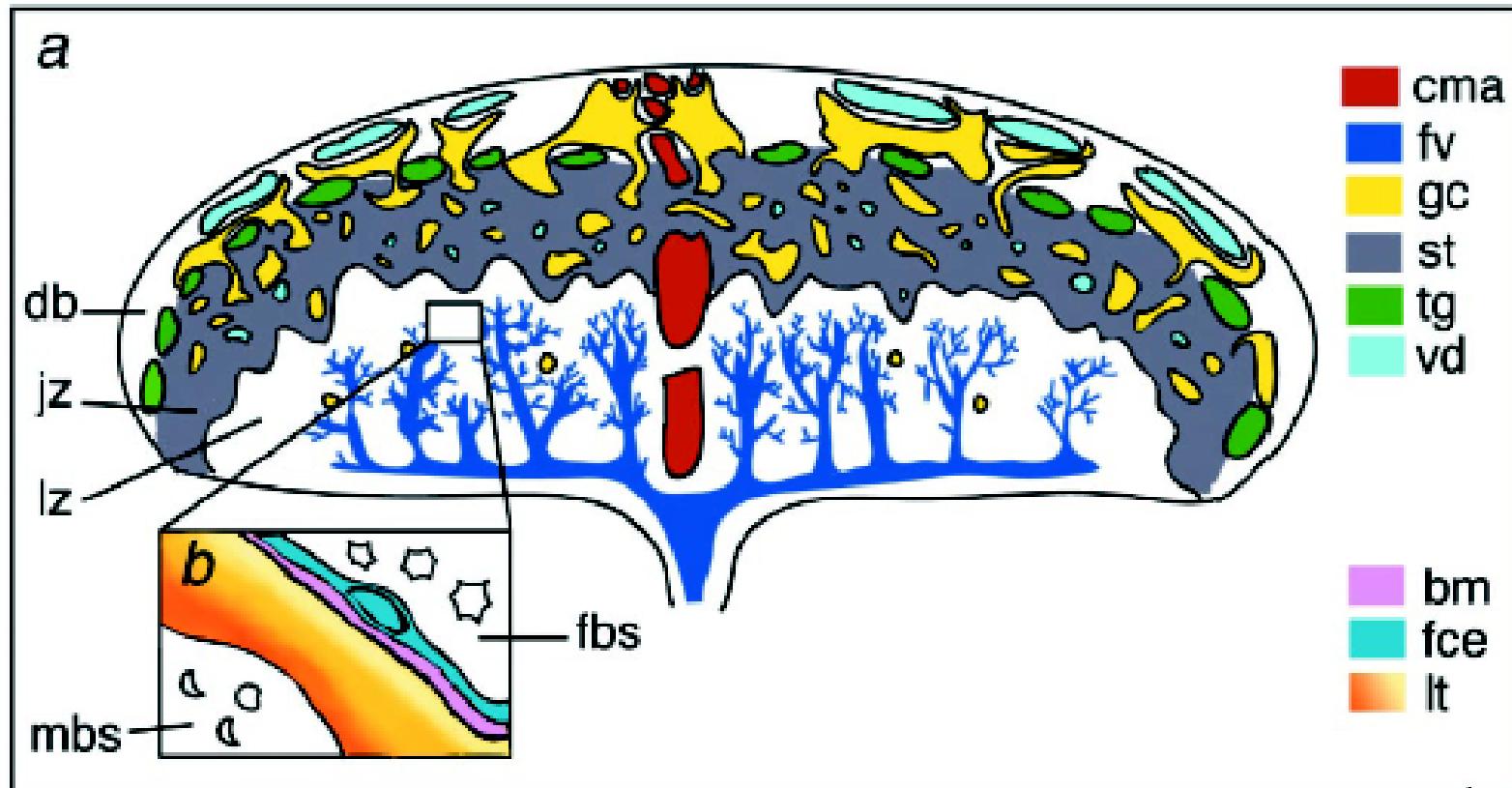
Primary placental effect: early GR of placenta, normal fetal Igf2 levels, catch-up growth

Why late fetal growth effect?

Placenta is nutrient-limiting only at late stages

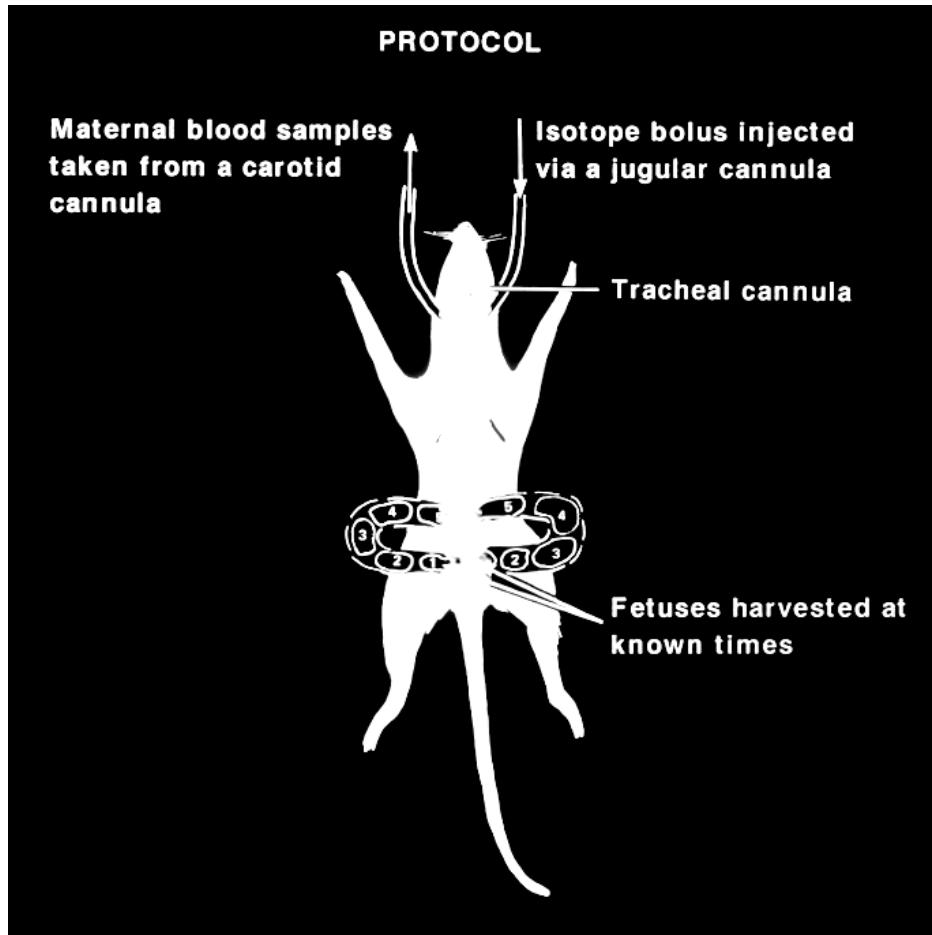
Increased efficiency of the small placenta

# Mouse placenta



Georgiades et al., PNAS 98:4522-7 (2001)

# Placental transfer assay



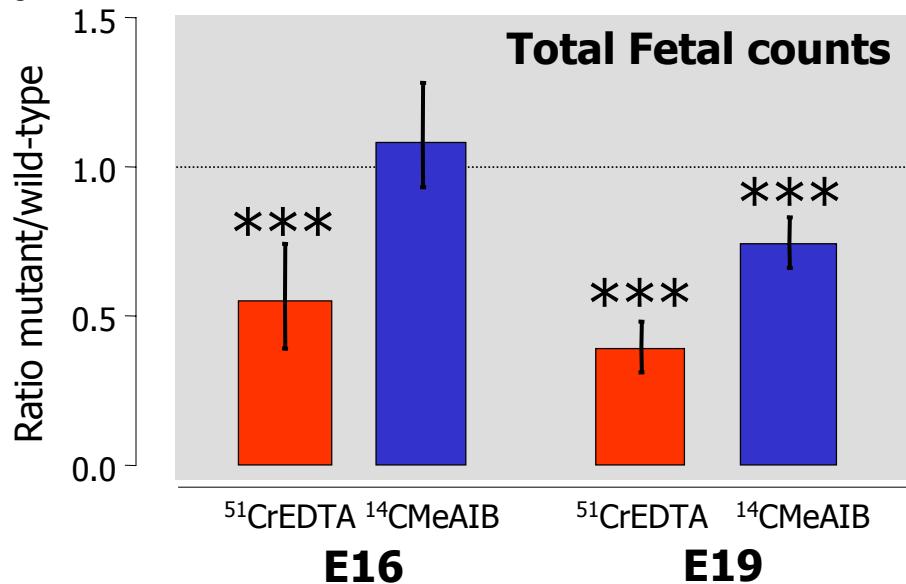
$^{51}\text{Cr}$  EDTA,  $^{14}\text{C}$  Inulin,  $^{14}\text{C}$  Mannitol - Passive diffusion

$^{14}\text{C}$  MeAIB – Active transport (amino acid)

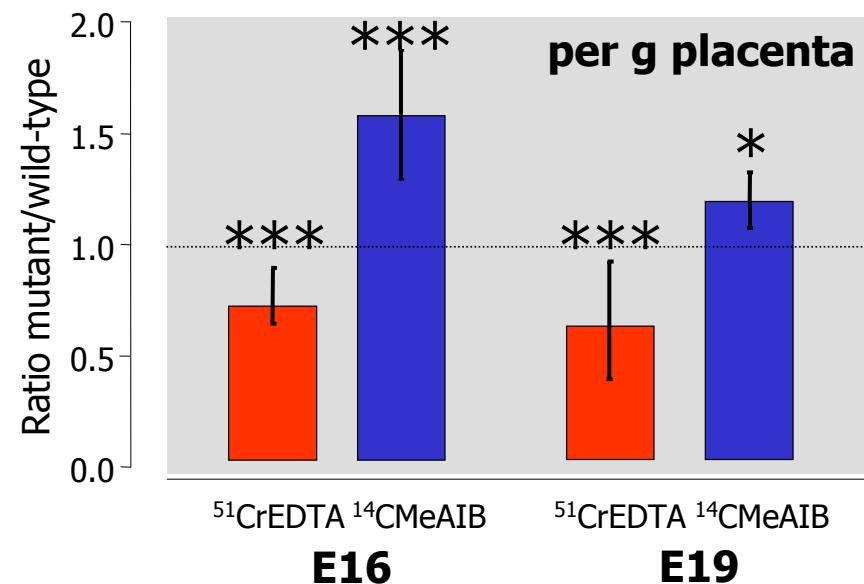
$^{14}\text{C}$  Glucose – Facilitated transport

# Passive diffusion is downregulated, and active amino acid transport upregulated

a



b



\*\*\* P<0.001

\* P<0.05

E16: ~ same fetal size

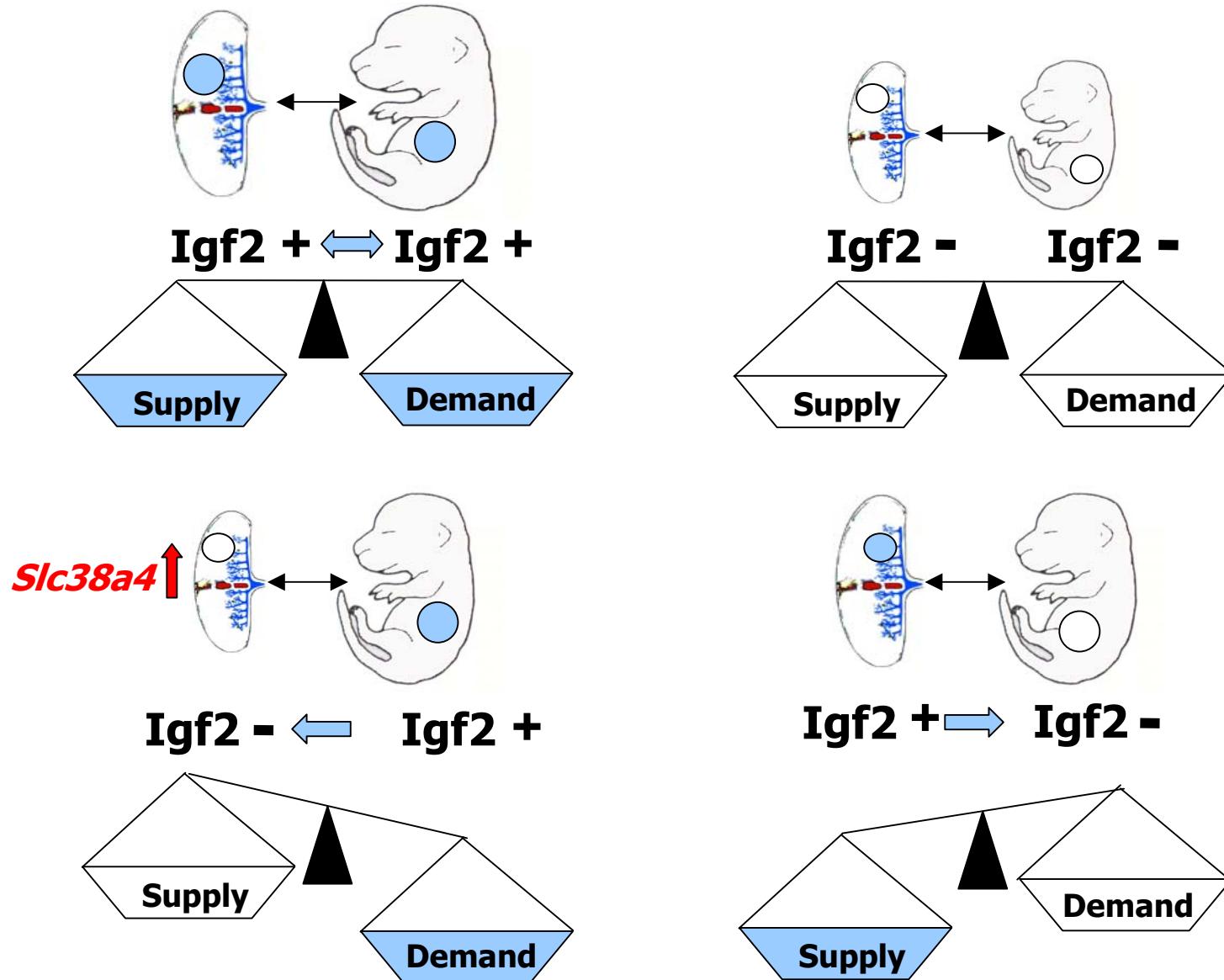
E19: ~ 22% smaller fetus

E16 & E19: placenta ~ 30% smaller

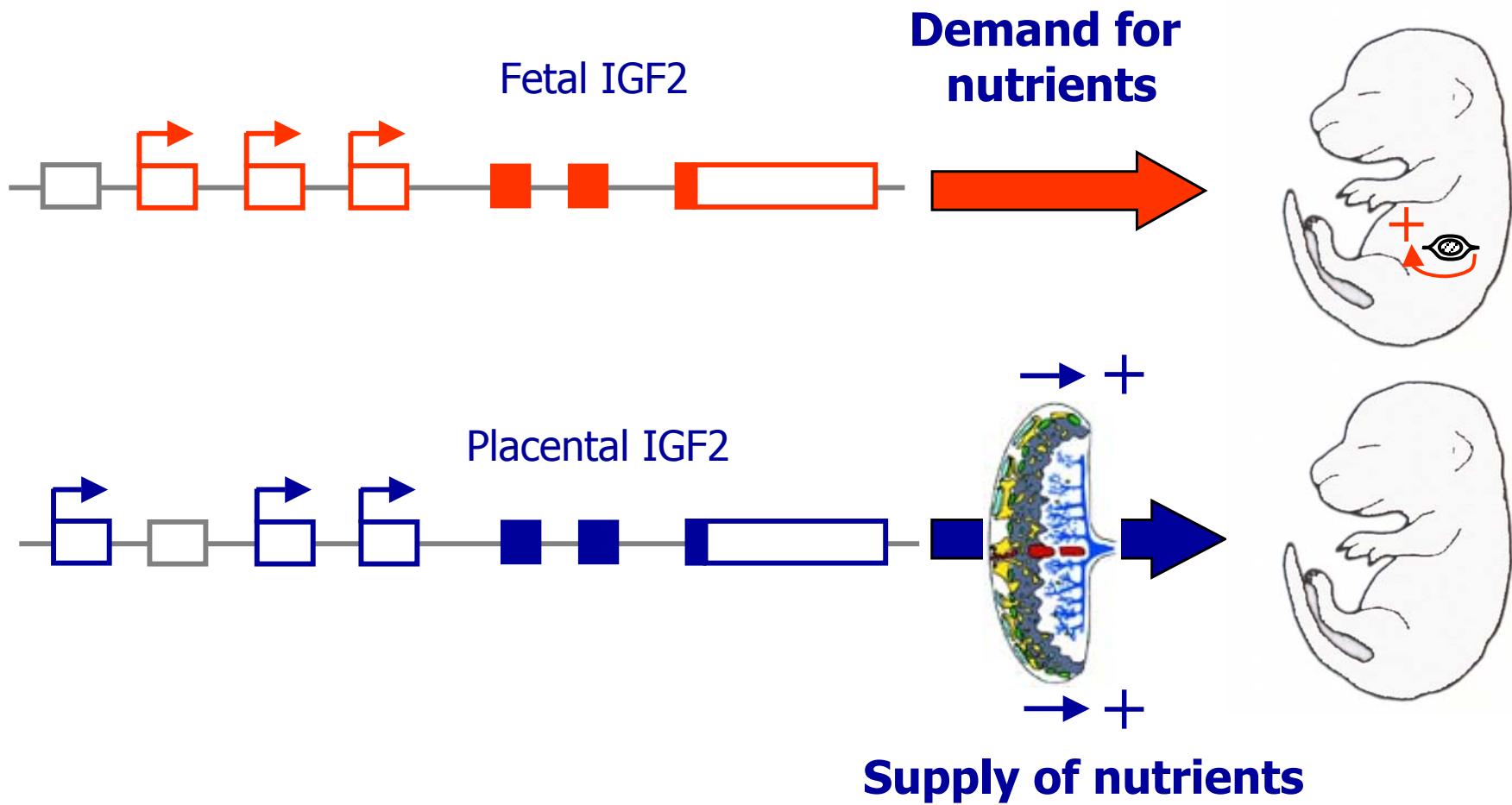
# **Placental-specific IGF2 is a major modulator of placental and fetal growth**

- Placental transfer assays establish a defect in passive diffusion, and initial upregulation in active transport which later decreases.
- This establishes a genetic model of IUGR and shows that placental IGF2 has a key role in nutrient transfer to the fetus
- Imprinted genes such as *Igf2* and *Slc38a4* may have linked functions and act as demand and supply signals

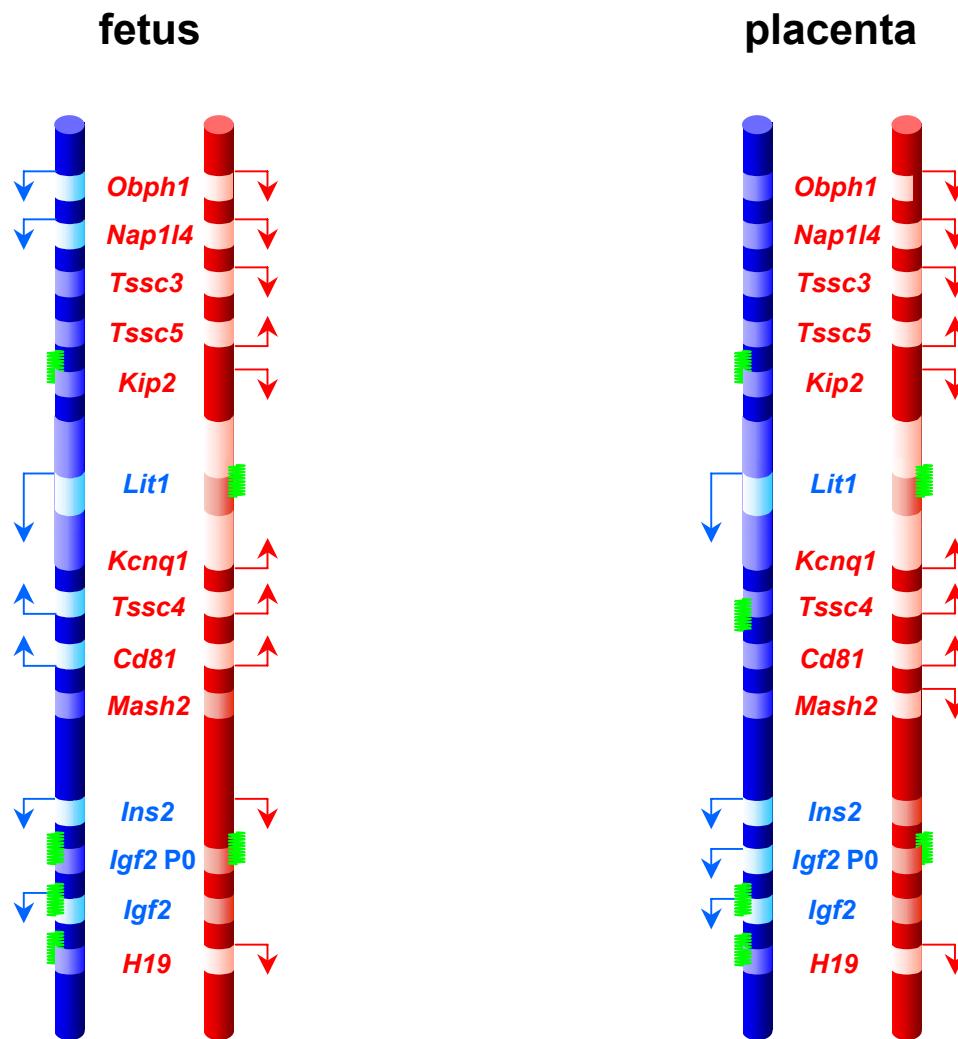
# Supply and demand signals



# Co-ordination of nutrient supply and demand by the imprinted Igf2 gene

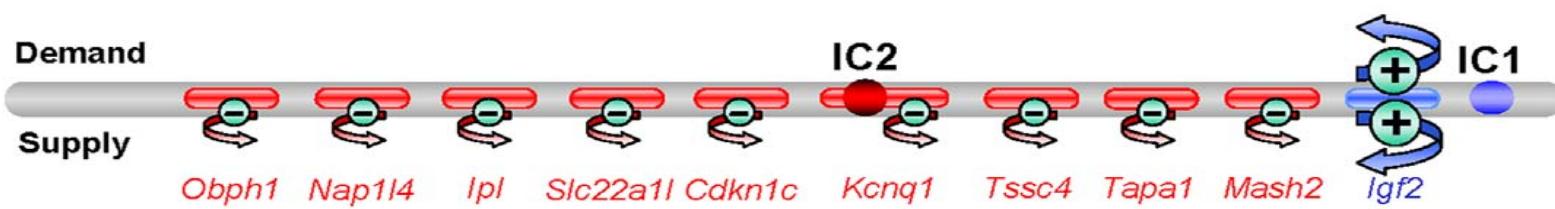


# Imprinting in embryo and placenta in the distal 7 cluster



Mitsuya K, 2003

# The supply and demand hypothesis of imprinting



# FUTURE CHALLENGES

- Trophoblast specific conditional knock-outs
- Integrate physiology & genome-wide transcriptional assays in mouse K.Os to identify nutrient demand and supply signals
- Interactions between imprinted genes in regulating nutrient supply to the mammalian fetus (e.g. *Igf2* and *Slc38a4*)
- Study of expression patterns of imprinted genes in extra-embryonic tissues; functional analysis
- Identification of novel placental-specific imprinted genes
- Role of epigenetic “mutations” in placental dysfunction

# Acknowledgements

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